PHAGE THERAPY BACK TO THE FUTURE!

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Antibiotic resistance is growing steadily; this is currently a worldwide problem. The economic costs are very high and the human impact is real. Environmental viruses called Bacteriophages are natural killers of bacteria. Experts agree that they are one of the strongest alternatives to antibiotics. They have been used empirically for many years in former Russian republics. Phagoburn is the first international multicentric study which aims to assess the safety and efficacy of two cocktails of bacteriophages to treat infected burn wounds either by *Escherichia coli* or by *Pseudomonas aeruginosa*. The 7th Framework Programme for Research and Development (European Community) funds this collaborative project. Henceforth, politicians should consider the future conditions of phage use in medicine. More human, economical and legal resources will be necessary for the next years. New public health rules will have to be written and anti-infection policies would have to be changed.

Antimicrobial resistance is a natural phenomenon in microbes, but the unrestrained use of antibiotics accelerates its occurrence. Treatment options for common infections are running out. All World Health Organization (WHO) regions but one report over 50% of resistant *Escherichia coli* strains to third generation cephalosporin (3GC) antibiotics. 3GC is one of the most potent class of antibiotics within our anti-bacterial arsenal. Worldwide, over 50% of *Klebsiella pneumonia* strains became resistant to 3GC. The risk of mortality is twice as high when patients are infected by an antibiotic resistant *Escherichia coli* strain.

Additional costs such as hospitalization, antibacterial therapy, medical care, etc. are increased by antibiotic resistant infections. In Europe, antibacterial resistance is responsible for 25,000 deaths per year and 2.5 million extra hospital days: the overall societal cost is estimated to reach €1.5 billion per year including €900 million for hospitalization days. In the United States, 23,000 deaths per year and 2 million illnesses are related to antibacterial resistance: direct and indirect societal costs are estimated to €20 and €35 billion, respectively *(1)*.

In January 2014, the executive board of the WHO, urged member states "to encourage and support research and development, including by academia and through new collaborative and financial models, to combat antimicrobial resistance and promote responsible use of antimicrobials, develop practical and feasible approaches for extending the lifespan of antimicrobial drugs and encourage the development of novel diagnostics and antimicrobial drugs".

Phage therapy is currently identified by many world experts as a promising way to fight resistance *(2–6)*.

Phages ecology and physiology

Phages are environmental viruses and natural bacterial parasites. They are the most important biomass on earth (7). Some of them are able to kill their specific targeted bacteria. They have been fighting bacteria for billions of years and help maintain a proper balance between host cells and bacterial populations within all living creatures. They are highly specific and only effective on bacteria. No toxic effects on mammalian (human) cells have been reported.

When a lytic phage finds its receptors on the bacterial wall, it hooks them up strongly. Then, the virus injects its genetic material inside the cytoplasm through the bacterial wall. The phage DNA (or RNA) sequence uses the bacteria to multiply and propagate. Bacteria are quickly destroyed (20 minutes), releasing many new phages able to strike new bacteria. The infection of bacteria by phages spreads very fast. As a consequence, the bacterial population is severely reduced, helping the human immune system get rid of it.

The bacteria destruction process may slow down if a strain

becomes resistant to the phage. To reduce such a risk one uses a "cocktail", i.e. a mix made of several different phages, to quickly decrease the bacterial inoculum, through various modes of action.

Historical clinical uses

Suspected in 1915 by Twort and discovered by d'Hérelle in 1917 at the Pasteur Institute (Paris) phages started to be used without any proper knowledge, leading to amazing but sometimes capricious results *(8)*. The discovery of penicillin in the late 1930s and the rise of antibiotics in the late 1970s led to their oblivion. It is only in the late 1980s, after the methicillin resistant *Staphylococcus aureus* (MRSA) superbug emerged in the United States, followed by the discovery of vials containing phages on Soviet Union soldiers (first Afghanistan war) that phage therapy was reborn in Western European countries.

Phages have been used in Tbilissi (Georgia) at the Georges Eliava's Institute since the 1930s and have been commercialized in Russian pharmacies by Microgen Co.® for decades. In the European Community, The Phage Therapy Centre in Poland offers phage therapy for compassionate treatment under the Declaration of Helsinki. Recently, the Queen Astrid Military Hospital in Brussels obtained approval from the Federal Government to perform such treatments. In France, phage therapy is scarcely used and only once all antibiotic drugs failed, but without any proper regulatory framework. In Washington and Oregon (USA), surgeons led by Dr Betty Kutter used Eliava Institute products on a case by case basis for treating diabetic foot infected ulcers. All these small initiatives show that phage therapy has to become a professionalized therapy, with approved manufacturing and clinical evaluation processes, before it can strengthen our anti-bacterial arsenal.

For years several western academic teams have been working on phage therapy – L Debarbieux (Pasteur Institute, Paris, France), J-P Pirnay (Queen Astrid Military Hospital, Brussels, Belgium), M Clockie (University of Leicester, UK), A Gorski (Institute of Immunology and Experimental Therapy, Wroclaw, Poland) and Betty Kutter at Evergreen State College (WA, USA), to name a few. By the turn of the century, various SME's Intralytix, Ampliphi Biosciences, Novolytics, Technophage, Micreos and Pherecydes Pharma, in collaboration with these public research institutions, started to look at phages with modern techniques - microbiology, electronic microscopy, molecular biology including phage genome sequencing and annotation.

Several phage products targeting various bacterial

Table 1: **Listings of companies**

Name of companies	Country	Web	Notes
AmpliPhi BioSciences Corporation	Australia	http://www.ampliphibio.com	Clinical trials against infections of the group « ESKAPE » on humans and among pets and livestock animals for MRSA and PYO
Biophage Pharma Inc	Canada	http://www.biophagepharma.net/index.php/en/	Biosensor division : dev. & commercialization of simple, accurate, highly sensitive biosensors based on phages / Therapeutic division dev. Phage therapies for human health.
Pherecydes Pharma	France	www.pherecydes-pharma.com	Development of phagetherapies for human health. EU funded PHAGOBURN clinical trial
Gangagen Inc.	India	www.gangagen.com	Developments of products against MRSA and PYO infections
Biotech Laboratories	Israel	www.biotec.com/index.asp	Rapid detection of rifampicine resistance in sputum positive for M.tb / Rapid detection of BK in human sputum
Micreos Food Safety	Netherlands	www.ebifoodsafety.com	Protection against LISTER in food preparation
CheilJedang Corp.	South Korea	www.cjj.co.kr	To protect chicken feed from Salmonella gallinarum et pullorum
Phico Therapeutics	UK	www.phicotherapeutics.co.uk	Bacteriophages for several bacteria : Listeria monocytogenes , M. tuberculosis), MRSA, MSSA
Novolytics	UK	www.novolytics.co.uk	Gels /MRSA / C. Difficile and products to decrease nasal portage of MRSA /gels for skin infections and medical devices
Biocontrol	UK	www.biocontrol-ltd.com	Cinical trials on otitis to treat PYO infections
Omnilytics	USA	www.phage.com	Development and use of lytic bacteriophages against tomato wilt disease
Intralytix	USA	www.intralytix.com	Decontamination and food additive against Escherichia coli O157:H7 in food preparation /Food additive against contamination by LISTER of uncooked food
Viridax Inc.	USA	www.viridax.com	Development of products against staphylococcal infections.
New Horizons Diagnostics Corporation	USA	http://www.nhdiag.com/phage.shtml	Enzybiotics: Phage Associated Enzymes (PAE) that act as antibiotics

infections such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Acinetobacter baumani*, *Clostridium difficile*, are being developed by SMEs, in various therapeutic domains: respiratory tract, intestinal tract, post-surgical, skin infections including burn wounds *(5,9–12)*, etc.

Standardized processes to produce this new class of therapeutic biological products, according to pharmaceutical Good Manufacturing Product (GMP) standards, are being developed. The clinical evaluation of phage cocktails within international, randomized, multicentric trials is ready to start in order to consolidate the historical data that have been developed by Eastern European countries.

Phagoburn

In burn wounds, the Phagoburn study aims at evaluating two phage cocktails, PP0121 and PP1131, for treating burn wound infections caused respectively by *Escherichia coli* or *Pseudomonas aeruginosa* bacteria, according to modern Western standards. The Phagoburn clinical trial is the first of its kind on a world scale.

It is a European collaborative project funded by the 7th Framework Programme for Research and Development (Health Programme). It has been launched on 10 June 2013 with a grant that amounts to €3.8 million for an overall budget of €5 million. Under the coordination of the French Ministry of Defence (Army Health Service in close collaboration with Pherecydes Pharma (French SME), Phagoburn gathers six other international burn treatment centres (France, Belgium, Switzerland). Starting in April 2015, phage therapy efficacy and safety will be evaluated through a phase II clinical multicentre study in accordance to Good Clinical Practices in France, Belgium and Switzerland. Prior to that, a second French SME, Clean Cells (France) has been in charge of adapting the genuine laboratory bioproduction process developed by Pherecydes Pharma into a true GMP (Good Manufacturing Practices) pharmaceutical manufacturing process to produce both clinical phage cocktails.

National drug regulatory agencies, ANSM (France), Swissmedic (Switzerland) and AFMPS (Belgium) are active supporters. Together with the European Medicine Agency (EMA), study on how to adapt the current regulatory framework for developing, testing and commercializing inert antibacterial molecules – antibiotics – for living biological therapies – bacteriophages. Phage diversity is outstanding and offers numerous way to fight anti-bacterial resistance, which needs to be taken in account within our western pharmacopeia guidelines. To make a parallel with flu vaccines: their valence can be adjusted each year to new viral strains. Phages offer a similar potential of adaptation to new form of bacterial resistance. But, our regulatory guidelines should allow phage addition or substitution within a therapeutic drug product, without starting from scratch the full process of clinical evaluation (7 to 10 years) after modifying a phage cocktail.

Phage and bacterial resistance

Our environment is full of phages, which can be associated in cocktails to target specific bacteria infections in specific areas, using various modes of action to reduce resistance occurrence.

Because of their bacterial specificity, phage cocktails are tailored to preserve the normal flora. When the human microbiota is maintained during an anti-bacterial treatment, it is more difficult for a phage resistant bacteria to emerge in such a competitive environment where other bacteria species are growing: this is a significant advantage compared to large spectrum antibiotics that destroy blindly all species of bacteria.

In addition, if a bacteria becomes resistant to phage, there is a fitness cost to acquire resistance: as a consequence the resistant strain becomes less virulent and can be more easily destroyed by the human immune system.

Future perspectives

Controlling phage therapy is necessary to avoid a misuse of a powerful therapy. However, patients without therapeutic options could benefit of this therapy, before market authorization is granted, under the control of specialized clinicians and the surveillance of national medicine agencies. As we learn from Ebola fever *(17)*, such an innovative therapy could be allowed by regulators before all the processes of clinical evaluation are completed, in regard to ethical considerations. Actually that issue may rise up quickly from patients infected by *E. coli* or *P. aeruginosa* resistant strains (they are very common bacteria), once the Phagoburn clinical trial is started.

As with antibiotics, phages could be used in animal farming to limit infectious diseases *(18)* and to boost productivity. However, politicians may have a key role in balancing their use between human and animal applications. A lose control in farming may lead to the same blast of resistance as we observed with antibiotics. Unwillingly selecting bacterial strains that are both resistant to antibiotics and phages could be the worst-case scenario. If a restricted use of phages to dead end patients is chosen, a restriction of phage use in industry/food/farming may be considered too.

From a fixed phage mix product today, to an evolving product where phages are added or substituted by others, one can even expect a product tailored to personalized medicine: phage preparations could be quickly and easily prepared for a local hospital infection or a food bacteria poisoning epidemic. But, the current regulatory framework in western countries is not tailored for that.

The association of phages with antibiotics could increase both product potencies. For instance, some phages are able to digest bacterial slime (biofilm), where most antibiotics are unable to reach the "encysted" pathogenic bacteria. Once the biofilm is loosen up, antibiotics may be able to kill the bacteria. Biofilms are commonly found on prosthesis.

Several actors are currently involved in the challenge of finding the right place for phage therapy in our future medicine arsenal. Patients infected by antibiotic multi-drug resistant strains expect an efficient anti-bacterial treatment to improve their life conditions and expectancy. SMEs are developing the pharmaceutical products. Regulators are ready to support desperately needed new anti-bacterial innovations. Medical teams hope that phages could push away the impact of antibiotic resistance on mortality related to bacterial diseases *(13–16)*. Certain European deputies and senators have started to advocate for phage therapy. However, more support is needed to develop bacteriophage collections, product formulation, high standard clinical trials and to adapt regulations.

Politicians have a key role to play.

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Both authors are coordinating a European Community funded project (FP7) to develop new bioproduction standards for phage drug manufacturing under the European pharmacopeia umbrella.

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